

- determining a distribution function of the number of photon counts measured per time interval,
- determining a distribution function of specific brightness of the particles based on the distribution function of the number of photon counts measured, by finding out the model of the sample yielding the closest fit between the experimentally determined and an expected distribution function of the number of photon counts, wherein the expected distribution function of the number of photon counts is calculated using characteristics of a spatial brightness function.

30. The method according to claim 29, wherein radiation from particles in one or more measurement volume(s) is measured.
31. The method according to claim 30, wherein the characteristics of the spatial brightness function are values of volumes of sections of the measurement volume corresponding to a selected set of values of the spatial brightness.
32. The method according to claim 31, wherein the volumes are considered as variables depending on modeling parameters of the spatial brightness function.

33. The method according to claim 32, wherein pinhole dimensions and convergence angle of an incident laser beam are modeling parameters of the spatial brightness function.
34. The method according to claim 32, further comprising the determination of the values of the parameters which yield the closest fit between the experimentally determined and the calculated expected distribution of the number of photon counts.
35. The method according to claim 29, wherein the detection means is part of a confocal microscopic set-up further having:
  - at least one microscope objective having an image plane for both focusing an incident laser beam and collecting radiation emitted, scattered and/or reflected by the particles of the sample,
  - a dichroic mirror,
  - a pin-hole in the image plane of the microscope objective,
  - and data acquisition means.
36. The method according to claim 35, wherein the confocal microscopic set-up further comprises means for scanning and/or moving the sample.

37. The method according to claim 35, wherein the at least one microscope objective has a numerical aperture  $\geq 0.9$ .
38. The method according to claim 35, wherein dimensions of the pinhole are used as a modeling parameter of the spatial brightness function.
39. The method according to claim 35, wherein the incident laser beam has a convergence angle used as a modeling parameter of the spatial brightness function.
40. The method according to claim 29, wherein the particles are molecules, macromolecules, dyes, molecular aggregates, complexes, vesicles, cells, viruses, bacteria, beads, or mixtures thereof in solids, liquids or gases
41. The method according to claim 29, wherein the particles can be grouped into particle species distinguished by their specific brightness.
42. The method according to claim 41, wherein at least one of the particle species is luminescent.
43. The method according to claim 41, wherein at least one of the particle species is luminescently labeled.

44. The method according to claim 41, wherein at least one of the particle species is fluorescent.
45. The method according to claim 42, wherein luminescence properties of the particle species are varied by conjugating the particle species with a first molecule capable of binding to a luminescently labeled second molecule.
46. The method according to claim 45, wherein (a) the first molecule is biotin and the luminescently labeled second molecule is avidin or streptavidin or (b) the first molecule is avidin or streptavidin and the luminescently labeled second molecule is biotin.
47. The method according to claim 45, wherein the first molecule is a (6xHis)tag and the second molecule is a luminescently labeled Ni-NTA-derivative.
48. The method according to claim 42, wherein luminescence properties of the particle species are changed by energy transfer, in which energy absorbed by the particle species is transferred upon close contact to a luminophore of an acceptor and subsequently emitted.
49. The method according to claim 29, wherein the particles carry binding sites for luminescent particles.

50. The method according to claim 30, wherein the measurement volume is less than the sample volume.
51. The method according to claim 50, wherein the measurement volume is  $\leq 10^{-12}$  l.
52. The method according to claim 50, wherein the particles move into and out of the measurement volume during measuring.
53. The method according to claim 50, wherein the particles are optically scanned.
54. The method according to claim 30, wherein the particles move into and out of the measurement volume during measuring.
55. The method according to claim 30, wherein the particles are optically scanned.
56. The method according to claim 30, wherein the measurement volumes are arranged two-dimensionally.

57. The method according to claim 56, wherein the measurement volumes are arranged on a membrane or a sheet having wells.
58. The method according to claim 30, wherein the measurement volumes are arranged linearly.
59. The method according to claim 58, wherein the measurement volumes are arranged in a capillary system.
60. The method according to claim 30, wherein the measurement volume is restricted by the use of elements of near field optical microscopy, or elements of near field optical microscopy in combination with conventional microscopy optics.
61. The method according to claim 44, wherein fluorescence is induced using multiple photon excitation.
62. The method according to claim 41, wherein parameters of the spatial brightness function characteristic for an optical equipment are determined by measuring numbers of photon counts, in a repetitive mode, from radiation emitted, scattered and/or reflected from a single particle species.

63. The method according to claim 41, wherein the concentration and/or specific brightness of at least one particle species is determined.
64. The method according to claim 29, wherein the distribution of the number of photon counts is fitted using a priori information on the sample.
65. The method according to claim 29, wherein the distribution of the number of photon counts is processed by applying an inverse transformation with linear regularization and/or constraints.
66. The method according to claim 29, wherein the detection means has experimental parameters, including dead time and after pulsing probability, determined by measuring, in a repetitive mode, numbers of photon counts per defined time interval while the detection means is exposed to light of constant intensity or high frequency laser pulses.
67. The method according to claim 29, further comprising determination of a background count rate of an equipment.
68. The method according to claim 29, wherein the time interval is on average smaller than the characteristic correlation time of radiation intensity fluctuations.

69. The method according to claim 29, wherein the time interval yields on average more than one photon count per particle of the sample.
70. The method according to claim 29, wherein the time interval yields on average one to ten photon counts per particle of the sample.
71. The method according to claim 30, wherein the size of the measurement volume comprises on average no more than a few particles.
72. The method according to claim 29, wherein at least one particle of the sample is statistically analyzed in terms of its specific brightness which may fluctuate or change non-stochastically.
73. The method according to claim 31, wherein the particles can be grouped into particle species distinguished by their specific brightness.
74. The method according to claim 73, wherein the distribution of the number of photon counts emitted, scattered and/or reflected by single species from a spatial section of constant brightness and detected by an ideal detector is compound Poissonian.